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III MATERIALS AND METHODS

2.0 Introduction:

The laboratory materials and different methods used in the present investigation were described in brief in this chapter.

2.1 Material

2.1.1 Selection of Larvivorous fishes :

Follwing Larvivorous fishes were selected for this research work

1) Guppy (*Poecilia reticulata,* peters)
2) Indian freshwater glass fish (*Chanda ranga)*
3) Indian freshwater topminnows (*Puntius sophore)*

1) Guppy (*Poecilia reticulata,* peters):

The guppy (*Poecilia reticulat*), also commonly known as guppy was one of the most popular freshwater aquarium fish species in the world. The guppy was also called as the millionfish. Robert John Lechmere Guppy had discovered this tiny fish in Trinidad in 1866, and the fish was named Girardinus guppi in his honour by Albert C.L.G. Gunther later that year. However, the fish had previously been described in America. Although Girardinus guppy is now considered a junior synonym of *Poecilia reticulata*, a common name "guppy" still remainred. Over time guppies have had been given a variety of taxonomic names, although *Poecilia reticulata*, was the name currently considered to be valid. The guppies were included as member of the live bearing family. It

preferred hard water aquarium and can withstand high levels of salinity (chervinski, 1984). It's most famous characteristic was it's propensity for breeding and it can breed in both fresh water and marine aquariums. The guppies had reported for complex networks by choosing social partners and remembering them.

"Imagine possessing something unique, something that you alone have it in the world. It doesn't come to you often but with guppies it is possible. Some people just think of guppy as a very cheap fish or prey for bigger fishes. However, with this cheap and insignificant fish you can make wonders!"

-wikimedia Foundation US

Scientific classification-

Kingdom	-	Animalia
Phylum	-	Chordata
Class	-	Actinopterygii
Order	-	Cyprinodontiformes
Family	-	Poeciliidae
Gunus	-	*Poecilia*
Species	-	*reticulata*

Natural habitat-

The wild, original guppy is native to central America, as well as Trinidad and Tobago, northern south America. Today, many guppies are found in Asia, especially Singapore, where many fancy strains are breed in large fish forms and shipped to pet stores all over the world.

***Chanda ranga*- (Ambassis)**

It is also called *Ambassis.* Two species, *Ambassis nama* and *Ambassis ranga* are widely distributed in the stagnances of Krishna river. They are of small size and translucent fishes. The body is covered with thin oblong glossy tightly overlapping cycloid scales. First dorsal fin spiny but second one smooth, the *Chanda ranga* species was rarely observed in the fisherman's catch. It is column feeder and recides in deeper zone of river stagnance. They are mainly cyclopsivorous but can enjoy mosquito larvae also. The mouth is superior, siphon shaped with elongated lower jaw – a speciality of Larvivorous fishes (Khanna, 1985)

Scientific classification-

Kingdom	-	Animalia
Phylum	-	Chordata
Family	-	Percidae
Gunus	-	*Chanda*
Species	-	*ranga*

2) *Puntius sophore* (Ham)

They are popularly called "carp minnows or topminnows". It measures about 10-12 cm in length and relatively smaller than *Puntius chola.* It has golden operculum and prominent caudal black spot in male only, female may have black spot which disappears during sexual maturation. Due to their tiny size, they can swim through submerged weeds and can attack on the aquatic insect larvae and rotifers.

Puntius sophore inhabits rivers, streams and ponds in plains and sub mountain regions. These are very plentiful shoaling fish. These fishes remains small in domestic aquaria and becomes matured at convenient size 10 to 12 cm.

Scientific classification-

Kingdom	-	Animalia
Phylum	-	Chordata
Class	-	Actinopterygii
Order	-	Cyprinodontiformes
Family	-	Poeciliidae
Gunus	-	*Puntius*
Species	-	*sophore*

2.1.2 Maintenance of Larvivorous fishes in aquarium :

For the study of histology and histochemistry of gonads in larvivorous fishes, the following fishes were selected for the histomorphological studies-

1) Guppy (*Poecilia reticulata,* peters)

2) Indian freshwater glass fish (*Chanda ranga)*

3) Indian freshwater topminnows (*Puntius sophore)*

These fishes were analyzed for their sexual cycle, histological and histochemical changes in the gonads. The continues live bearing exotic guppy fishes *Poecilia reticulata* were selected for the maintenance in the aquarium and in the natural fresh water for their propogation and hence maintained in the aquarium and the ponds.

2.1.3 Maintenance of Guppy in aquarium:

One of the most popular freshwater aquarium fish is the guppy (Gupta and Gupta, 2006). Guppies were noted for having different colors and patterns that are very unique and beautiful. No two guppies are exactly alike. Guppies are fascinating to watch and they are remarkably easy and enjoyable to care for.

The live guppies were brought to laboratory from sewage swamps of near by kirloskar steel industry. They were kept in aquarium with proper maintenance. The fishes were regularly fed with baby shrimp bran meal and powdered crustacean larvae (ver. Sukat). The fishes were observed for their strong affinity towards organic sewage particles were fed with live chironomous larvae and mosquito larvae, from the eutrophid ditches and observed for their feeding potential of the larvae. Only care was taken that the other big predatory fishes were kept away from the guppy.

However, flourishing reproductive activity was not observed in a confined laboratory aquarium therefore these were maintained in the natural fresh water pond in our college garden nearby .

2.1.4 Maintenance of Guppy in fresh water ponds :

A few live guppies collected from near by sewage swamps were kept in the freshwater rectangular pond (10' X 5' X 4')

The circular type of pond like that of circular chinise hatchary was also constructed in our college garden, only care was taken that the ponds were kept young all the while. The abundant submerged aquatic weeds such as hydrilla, spirogyra, oedogonium and few rhizomes of nymphea were added in the pond, as for a hiding places for gravid females and fries. Even such a pond was observed for their rich microscopic phytoplankton and zooplankton. The indicator of such a fertile pond was basal colonies of vorticella which could often tasted by gravid females. The guppies were proliferated in thousands and millions. With the help of team of students in college, the guppies were redistributed in the water reservoirs of remote corner villages in the Palus Tahsil of Sangli district (MS).

2.1.5 Maintenance of *Chanda ranga* in the aquarium:

The tiny glossy freshwater stagnant fishes were brought from the wells, and the irrigation channels in live forms. These were maintained in a separate freshwater aquarium for long period. The fishes were observed for their mosquito larvae feeding potential and other competitive behavior if any.

They were rarely found in the shallow river water, However abundant population of *Chanda ranga* was noticed in the stagnance of brackish water, could be helpful for biological control of malaria in coastal marshy localities.

2.1.6 Maintenance of *Punitus sophore* in the aquarium:

The Indian freshwater topminnow *Puntius sophore* was abundantly found in confide freshwater of Yerala and Krishna rivers. It was observed that, the fish is neglected from the freshwater fishing programme though promising for their larvivorous feeding habit. The live minnows were brought to laboratory and maintained for observation of feeding potential and selectivity in their feeding habit.

2.1.7 Artificial fish meal :

The guppy fishes were maintained on the fish meal, prepared from dried, coarsely crushed crustacean larvae. In a local market so called "Sukat" is nothing but dried oceanic crustacean larvae. The rate of reproduction in guppy was accelerated due to crushed dried form of crustacean zooplankton as a fish meal. The artificial fish meal for *P. reticulata* and *Chanda ranga* is given in the form of baby shrimp bran, crushed earthworms, plentiful larvae, rotifers from ditches.

2.2 Methods :

2.2.1 Fresh dissections of Larvivorous fishes, Photomicrography, fixation of gonads:

Guppy (*Poecilia reticulata)*

A full grown males and females of *P. reticulata* were obtained from the freshwater garden pond being constructed in our college. The fishes were dissected with the help of pointed needles, razor and other

instruments under the binocular microscope in 0.3% saline. Immediately the fishes were dissected out for their testes and ovaries. The photomircorgraphs were also taken under the microscope. Then the tissues were fixed in cold 2% calcium acetate in 10% formalin (CAF). The fixed tissues were kept at 4^0C for 24hr. then the tissues were washed in clean running water for 24hrs. then the tissues were dehydrated in series of ascending grades of alcohol (30%, 50%, 70%, 90% and absolute), embedded in paraffin was at 58^0C and sections were cut at 5-7 micron. After proper spreading the sides were processed for histological and histochemical techniques.

Chanda ranga –

During the histological and histochemical studies matured males and females of *Chanda ranga* were brought from the fishermen's , these fishes were collected from the fresh catch during their sexual inactive, prebreeding, active breeding and the post breeding period.

The respective matured males and females were quickly dissected for their ovaries and testes. The tissues were fixed in cold CAF (2% calcium acetate + neutral formalin) for 24hrs, at 4^0C . After prolonged fixation of 24hrs, the tissues were washed in chilled water and dehydrated through ascending grades of alcohols (30%, 50%, 70%, 90%, absolute) and brought to xylene. If at all in a delay or time laps the tissues were embedded in paraffin was at 58^0C and sections were cut at 5 to 7 micron.

After proper spreading the slides were processed for histological and histochemical techniques.

***Puntius sophore* (Ham)-**

The *Puntius sophore* was observed their seasonal breeding activity. They showed active breeding period during the rainy season (June to September). They were observed for their strong up stream migration for the breeding activity. The mature males and females were collected from the local fishermen or they were brought in a live form in a polythene bag during their sexual quicent prebreeding, active breeding and post breeding periods.

The fresh live fishes were dissected for their testes and ovaries. The anatomical structure of tests and ovaries was observed.

The freshly dissected testes and ovaries were kept in a freeze, then fixed into cold CAF (2% calcium acetate and 4% neutral formalin) for 24hrs. After prolonged fixation, tissues were thoughraly washed in chilled, distilled water or running tap water for 24hrs. Then tissues were dehydrated in a series of ascending grades of alcohols (30%, 50%, 70%, 90% and absolute) and brought to xylene. Then the tissues embedded in paraffin wax at 58^0C , and sections were cut at 5 to 7 micron. After proper spreading the slides were processes for histological and histochemical techniques.

2.2.2 Histological and Histochemical Methods :

Following histological and histochemical methods were employed for this research work.

1) Routine HE technique
2) Alcian Blue (AB), PH.1. (Nalwade 1975; Scott et al 1964; Lev And Spicer 1964) .
3) Alcian Blue (AB), PH 2.5 (Nalwade, 1975; Mowry 1956; Scott and Dorling 1965; Quantarell, G. 1963).
4) Periodic acid schiff (PAS) technique (Schabodasch 1947; McManus 1946).